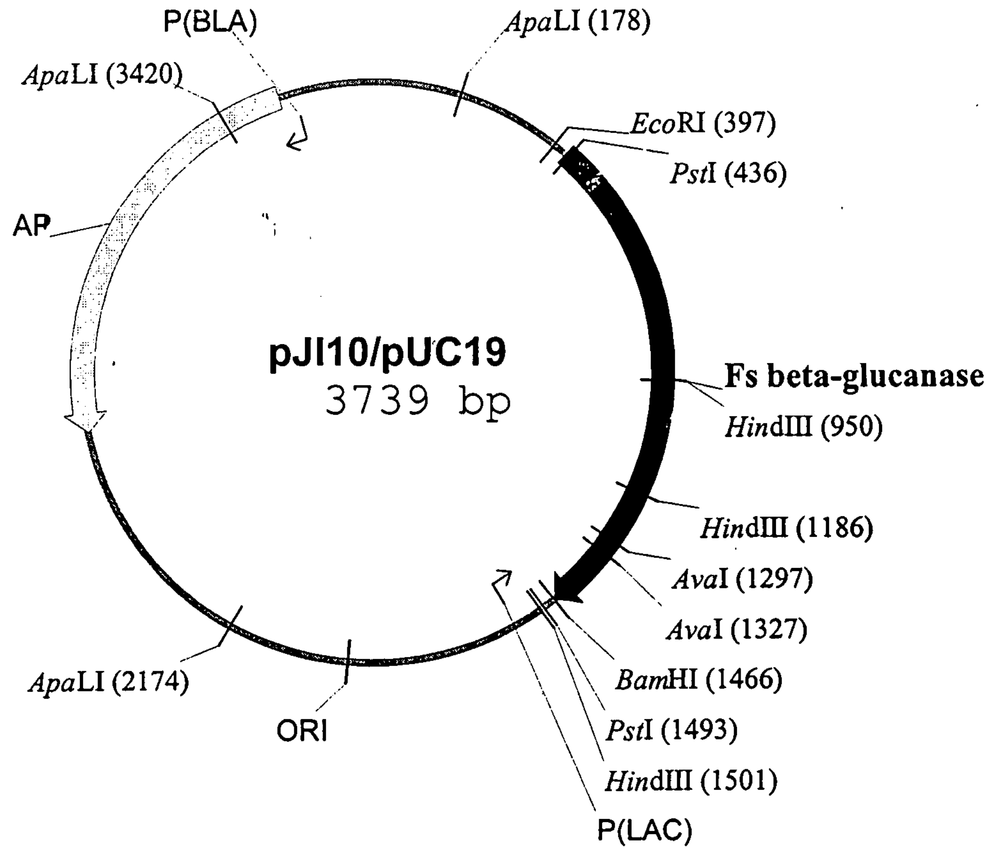
Fig.1



pJI10/pUC19
3739 bp
P(BLA)
ApaLI (3420)
AP
ApaLI (178)
EcoRI (397)
PstI (436)
Fs beta-glucanase
HindIII (950)
HindIII (1186)
AvaI (1297)
AvaI (1327)
BamHI (1466)
PstI (1493)
HindIII (1501)
P(LAC)
ApaLI (2174)
ORI

Fig. 2 The amino acid sequence in Fig. 2 is SEQ ID NO: 1
the DNA sequence in Fig. 2 is SEQ ID NO: 4

```
ATGGTTAGCGCAAAGGATTTTAGCGGTGCCGAACTCTACACGTTAGAAGAAGTTCAGTAC
 M  V  S  A  K  D  F  S  G  A  E  L  Y  T  L  E  E  V  Q  Y    20

GGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGTTCCATGTTC
 G  K  F  E  A  R  M  K  M  A  A  A  S  G  T  V  S  S  M  F    40

CTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTGGATATTGAA
 L  Y  Q  N  G  S  E  I  A  D  G  R  P  W  V  E  V  D  I  E    60

GTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAGGCCGGCGCA
 V  L  G  K  N  P  G  S  F  Q  S  N  I  I  T  G  K  A  G  A    80

CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCTTTCCACACC
 Q  K  T  S  E  K  H  H  A  V  S  P  A  A  D  Q  A  F  H  T   100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC
 Y  G  L  E  W  T  P  N  Y  V  R  W  T  V  D  G  Q  E  V  R   120

AAGACGGAAGGTGGCCAGGTTTCCAACTTGACAGGTACACAGGGACTCCGTTTTAACCTT
 K  T  E  G  G  Q  V  S  N  L  T  G  T  Q  G  L  R  F  N  L   140

TGGTCGTCTGAGAGTGCGGCTTGGGTTGGCCAGTTCGATGAATCAAAGCTTCCGCTTTTC
 W  S  S  E  S  A  A  W  V  G  Q  F  D  E  S  K  L  P  L  F   160

CAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAAGGCGGCAGC
 Q  F  I  N  W  V  K  V  Y  K  Y  T  P  G  Q  G  E  G  G  S   180

GACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGCTGGGGCAAG
 D  F  T  L  D  W  T  D  N  F  D  T  F  D  G  S  R  W  G  K   200

GGTGACTGGACATTTGACGGTAACCGTGTCGACCTCACCGACAAGAACATCTACTCCAGA
 G  D  W  T  F  D  G  N  R  V  D  L  T  D  K  N  I  Y  S  R   220

GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAACGGCCAGGTT
 D  G  M  L  I  L  A  L  T  R  K  G  Q  E  S  F  N  G  Q  V   240

CCGAGAGATGACGAACCTGCTCCG
 P  R  D  D  E  P  A  P                                       248
```

Fig. 3

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Fig. 3 The amino acid sequence in Fig. 3 is SEQ ID NO: 2

The DNA sequence in Fig. 3 is SEQ ID NO: 5

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ATGGTTAGCGCAAAGGATTTTAGCGGTGCCGAACTCTACACGTTAGAAGAAGTTCAGTAC
 M  V  S  A  K  D  F  S  G  A  E  L  Y  T  L  E  E  V  Q  Y   20

GGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGTTCCATGTTC
 G  K  F  E  A  R  M  K  M  A  A  A  S  G  T  V  S  S  M  F   40

CTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTGGATATTGAA
 L  Y  Q  N  G  S  E  I  A  D  G  R  P  W  V  E  V  D  I  E   60

GTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAGGCCGGCGCA
 V  L  G  K  N  P  G  S  F  Q  S  N  I  I  T  G  K  A  G  A   80

CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCTTTCCACACC
 Q  K  T  S  E  K  H  H  A  V  S  P  A  A  D  Q  A  F  H  T  100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC
 Y  G  L  E  W  T  P  N  Y  V  R  W  T  V  D  G  Q  E  V  R  120

AAGACGGAAGGTGGCCAGGTTTCCAACTTGACAGGTACACAGGGACTCCGTTTTAACCTT
 K  T  E  G  G  Q  V  S  N  L  T  G  T  Q  G  L  R  F  N  L  140

TGGTCGTCTGAGAGTGCGGCTTGGGTTGGCCAGTTCGATGAATCAAAGCTTCCGCTTTTC
 W  S  S  E  S  A  A  W  V  G  Q  F  D  E  S  K  L  P  L  F  160

CAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAAGGCGGCAGC
 Q  F  I  N  W  V  K  V  Y  K  Y  T  P  G  Q  G  E  G  G  S  180

GACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGCTGGGGCAAG
 D  F  T  L  D  W  T  D  N  F  D  T  F  D  G  S  R  W  G  K  200

GGTGACTGGACATTTGACGGTAACCGTGTCGACCTCACCGACAAGAACATCTACTCCAGA
 G  D  W  T  F  D  G  N  R  V  D  L  T  D  K  N  I  Y  S  R  220

GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAACGGCCAGGTT
 D  G  M  L  I  L  A  L  T  R  K  G  Q  E  S  F  N  G  Q  V  240

CCGAGAGATGACGAACCTGCTCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTC
 P  R  D  D  E  P  A  P  N  S  S  S  V  D  K  L  A  A  A  L  260

GAGCACCACCACCACCACCACTGA
 E  H  H  H  H  H  H  *                                      267
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Fig. 4

Table 1. Comparison of kinetic properties of *F. succinogenes* and *B. subtilis* 1,3-1,4-β-D-glucanases

Enzyme	Specific activity (U/mg)	k_{cat} ($^{-1}$s)	Opt. Temperature (°C)	Opt. pH
Wild-type	2065 ± 82	1296 ± 51	50 (at pH 6.0)	6.0-8.0
TG-Glucanase	7980 ± 341	3695 ± 158	50 (at pH 6.0)	6.0-8.0
PCR-TF-Glucanase	7833 ± 334	3911 ± 166	50 (at pH 6.0)	6.0-8.0
Lichanase (Megazyme)	118[a]	47.2[a]	60 (at pH 6.5)[a]	6.5-7.0[a]
	82.6 ± 0.96	33.0 ± 0.38	55 (at pH 7.0)	

The kinetics was performed with lichenan (6mg/mL) as substrate in 50 mM citrate buffer (pH 6.0) or in 50mM phosphate buffer (pH 7.0), and at optimum temperature as indicated.

[a]: Data was taken from *Megazyme* instruction brochure of lichenase. The kinetics was done with barley β-glucan (5mg/mL) as substrate.

Fig. 5

Table 3. Reactivation of PCR-TF-glucanase at 25 °C after heat treatment

Heat treatment	Recovery time (min)	Relative activity (%)
90 °C, 10 min	10	68
	20	81
90 °C, 30 min	10	61
	20	67
100 °C, 10 min	10	68
	20	72
100 °C, 30 min	10	55
	20	56

Fig. 6a The amino acid sequence in Fig. 6 is SEQ ID NO: 3

(part a) The DNA sequence in Fig. 6 is SEQ ID ~~ID~~ NO: 6

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ATGAACATCAAGAAAACTGCAGTCAAGAGCGCTCTCGCCGTAGCAGCCGCAGCAGCAGCC
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CTCACCACCAATGTTAGCGCAAAGGATTTTAGCGGTGCCGAACTCTACACGTTAGAAGAA
 L  T  T  N  V  S  A  K  D  F  S  G  A  E  L  Y  T  L  E  E   40

GTTCAGTACGGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGT
 V  Q  Y  G  K  F  E  A  R  M  K  M  A  A  A  S  G  T  V  S   60

TCCATGTTCCTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTG
 S  M  F  L  Y  Q  N  G  S  E  I  A  D  G  R  P  W  V  E  V   80

GATATTGAAGTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAG
 D  I  E  V  L  G  K  N  P  G  S  F  Q  S  N  I  I  T  G  K  100

GCCGGCGCACAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCT
 A  G  A  Q  K  T  S  E  K  H  H  A  V  S  P  A  A  D  Q  A  120

TTCCACACCTACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAG
 F  H  T  Y  G  L  E  W  T  P  N  Y  V  R  W  T  V  D  G  Q  140

GAAGTCCGCAAGACGGAAGGTGGCCAGGTTTCCAACTTGACAGGTACACAGGGACTCCGT
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CCGCTTTTCCAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAA
 P  L  F  Q  F  I  N  W  V  K  V  Y  K  Y  T  P  G  Q  G  E  200

GGCGGCAGCGACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGC
 G  G  S  D  F  T  L  D  W  T  D  N  F  D  T  F  D  G  S  R  220

TGGGGCAAGGGTGACTGGACATTTGACGGTAACCGTGTCGACCTCACCGACAAGAACATC
 W  G  K  G  D  W  T  F  D  G  N  R  V  D  L  T  D  K  N  I  240

TACTCCAGAGATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAAC
 Y  S  R  D  G  M  L  I  L  A  L  T  R  K  G  Q  E  S  F  N  260

GGCCAGGTTCCGAGAGATGACGAACCTGCTCCGCAATCTTCTAGCAGCGCTCCGGCATCT
 G  Q  V  P  R  D  D  E  P  A  P  Q  S  S  S  S  A  P  A  S  280

TCTAGCAGTGTTCCGGCAAGCTCCTCTAGCGTCCCTGCCTCCTCGAGCAGCGCATTTGTT
 S  S  S  V  P  A  S  S  S  S  V  P  A  S  S  S  S  A  F  V  300

CCGCCGAGCTCCTCGAGCGCCACAAACGCAATCCACGGAATGCGCACAACTCCGGCAGTT
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